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CONTENTS

Development and Validation of a Simultaneous HPTLC Method for the Estimation of Olmesartan medoxomil and Hydrochlorothiazide in Tablet Dosage Form

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Shah, *et al.*: Simultaneous HPTLC Estimation of Olmesartan and Hydrochlorothiazide

A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the estimation of olmesartan medoxomil and hydrochlorothiazide simultaneously in combined dosage forms. The stationary phase used was precoated silica gel $60F_{254}$. The mobile phase used was a mixture of acetonitrile:chloroform:glacial acetic acid (7:2:0.5, v/v/v). The detection of spots was carried out at 254 nm. The method was validated in terms of linearity, accuracy, precision and specificity. The calibration curve was found to be linear between 500 to 750 ng/spot for olmesartan medoxomil and 100 to 600 ng/spot for hydrochlorothiazide. The limit of detection and the limit of quantification for the olmesartan medoxomil were found to be 170 and 500 ng/spot, respectively and for hydrochlorothiazide 30 and 100 ng/spot, respectively. The proposed method can be successfully used to determine the drug content of marketed tablet formulation.

Key words: HPTLC estimation, olmesrtan medoxomil, hydrochlorthiazide

Olmesartan medoxomil is 2,3-dihydroxy-2-butenyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl]imidazole-5-carboxylate, cyclic 2,3-carbonate[1] and chemically hydrochlorothiazide is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide. Olmesartan medoxomil, a prodrug, is hydrolyzed to olmesartan during absorption from the gastrointestinal tract. Olmesartan is a selective AT_1 subtype angiotensin II receptor antagonist. Literature survey reveals that few HPLC and spectrophotometry methods are reported for the estimation of olmesartan medoxomil in the biological samples such as plasma[2-4]. So far no HPTLC method has been reported for the estimation of olmesartan medoxomil and hydrochlorothiazide in combined dosage forms. In the present investigation an attempt has been made to develop accurate and precise HPTLC method for the simultaneous estimation of olmesartan medoxomil and hydrochlorothiazide in combined dosage forms.

Olmesartan medoxomil and hydrochlorothiazide standard were procured as a gift sample from Sankyo Pharmaceuticals Ltd., Mumbai. Silica gel $60F_{254}$ TLC plates (10×10 cm, layer thickness 0.2 mm, E. Merck, Mumbai) were used as a stationary phase. All chemicals and reagents used were of analytical grade. Tablets containing olmesartan medoxomil (40 mg) and hydrochlorothiazide (12.5 mg) were procured from local pharmacy store (Benicar, Sankyo Pharmaceutical Ltd). A Camag HPTLC system comprising of Camag Linnomate V automatic sample applicator, Hamilton syringe (100 µl), Camag TLC Scanner 3, Camag WinCATS software, Camag Twin-trough chamber (10 x 10 cm) and ultrasonicator were used during study.

Olmesartan medoxomil and hydrochlorothiazide (25 mg) each were weighed accurately, dissolved and diluted with methanol to obtain the final concentration of 100 µg/ml of each drug. Twenty tablets were weighed accurately and ground to fine powder. Weight equivalent to 25 mg of olmesartan medoxomil and hydrochlorothiazide were transferred to conical flask and mixed with methanol. The solution was sonicated for 15 min. The extracts were filtered through Whatmann filter paper No. 41 and residue was washed with methanol. The extracts and washing were pooled and transferred to a 250 ml volumetric flask and volume was made up to 250 ml with methanol.

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Required dilutions were made to get 100 µg/ml of olmesartan medoxomil and hydrochlorothiazide.

TLC plates were prewashed with methanol. Activation of plates was done in an oven at 50^0 for 5 min. The chromatographic conditions maintained were precoated silica gel $60F_{254}$ aluminum sheets (10×10 cm) as stationary phase, acetonitrile: chloroform:glacial acetic acid (7:2:0.5, v/v/v) as mobile phase, chamber and plate saturation time of 30 min, migration distance allowed was 72 mm, wavelength scanning was done at 254 nm keeping the slit dimention at 5×0.45 mm. A deuterium lamp provided the source of radiation. Standard solutions of olmesartan medoxomil and hydrochlorothiazide (4 µl) were spotted and developed at constant temperature. Photometric measurements were performed in reflectance mode with Camag TLC scanner 3 using Win CATS software at 254 nm.

Aliquots of 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 µl of standard solution of olmesartan medoxomil and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 µl of hydrochlorothiazide were applied on the TLC plate (100 µg/ml of drug). TLC plate was dried, developed and analyzed photometrically as described earlier. The standard calibration curve was generated using regression analysis with Microsoft excel.

TABLE 1: METHOD VALIDATION PARAMETERS OF PROPOSED METHOD

Parameters	Values	
	Olmesartan medoxomil	Hydrochlo-rothiazide
Linearity range (ng/spot)	500-750	100-600
Correlation coefficient (r)	0.9991	0.9993
Regression equation (y=mx+c)		
Slope (m)	9.89	3.13
Intercept (c)	-3588.5	138.51
Limit of detection (LOD)	170 ng/spot	30 ng/spot
Limit of quantification (LOQ)	500 ng/spot	100 ng/spot
Precision (%CV)		
Repeatability of application (n=5)	0.12	0.77
Repeatability of measurement (n=5)	0.42	0.72

Six microlitres of sample solutions of the marketed formulation was spotted on to the same plate followed by development scanning. The analysis was repeated in triplicate. The content of the drug was calculated from the peak areas recorded.

The developed method was validated in terms of linearity, accuracy, limit of detection, limit of quantification, intra-day and inter-day precision and repeatability of measurement as well as repeatability of sample application[5].

The mobile phase consisting of acetonitrile: chloroform: glacial acetic acid (7:2:0.5, v/v/v) gave R_f values of 0.58±0.04 and 0.68±0.02 for olmesartan medoxomil and hydrochlorothiazide, respectively. The linear regression data (n=5, Table 1) showed a good linear relationship over a concentration range of 500-750 ng/spot and 100-600 ng/spot for olmesartan medoxomil and hydrochlorothiazide, respectively. The limit of detection and limit of quantification for olmesartan medoxomil was found to be 170 and 500 ng/spot and for hydrochlorthiazide, 30 and 100 ng/spot, respectively.

The intra-day precision was determined by analyzing standard solutions in the concentration range of 600 ng/spot to 750 ng/spot for olmesartan medoxomil and 200 ng/spot to 500 ng/spot for hydrochlorothiazide for 3 times on the same day while inter-day precision was determined by analyzing corresponding standards daily for 3 day over a period of one week. The intra-day and inter-day coefficients of variation were in the range of 1.30 to 1.52 for olmesartan medoxomil and 1.00 to 1.45 for hydrochlorothiazide. Repeatability of sample application was assessed by spotting 6 µl of drug solution 5 times on a TLC plate followed by development of plate and recording the peak area for 5 spots. The % RSD for peak area values of olmesartan medoxomil and hydrochlorothiazide were found to be 0.12 and 0.77, respectively. Repeatability of measurement of peak

TABLE 2: RECOVERY STUDIES OF OLMESARTAN MEDOXOMIL AND HYDROCHLOROTHIAZIDE

Brand name	Label claim mg / tablet	Total amount added (mg)	Amount recovered* (mg) ± SD	% Recovery ± SD	% Assay*
	Olmesartan medoxomil	40	39.61±0.06	99.03±0.06	
	40	60	60.02±0.01	100.04±0.01	99.29±0.60
Benicar		80	79.96±0.08	99.96±0.09	
		25	25.01±0.06	100.07±0.07	
	Hydrochlorothiazide	37.5	37.44±0.24	99.86±0.24	101.43±1.76
	12.5	50	50.57± 0.01	101.14±0.01	

*Each value is a mean±standard deviation of three determinations. Benicar is a brand of Sankyo Pharmaceutical Ltd.

area was determined by spotting 6 µl of olmesartan medoxomil and hydrochlorothiazide solution on a TLC plate and developing the plate. The separated spot was scanned five times without changing the position of the plate and % RSD for measurement of peak area of olmesartan medoxomil and hydrochlorothiazide were found to be 0.42 and 0.72, respectively. To confirm the specificity of the proposed method, the solution of the formulation was spotted on the TLC plate, developed and scanned. It was observed that the excipients present in the formulation did not interfere with the peaks of olmesartan medoxomil and hydrochlorothiazide.

Recovery studies of the drugs were carried out for the accuracy parameter. These studies were carried out at three levels i.e. multiple level recovery studies. Sample stock solution from tablet formulation of 100 µg/ml of was prepared. Dilutions were made and recovery studies were performed. Percentage recovery was found to be within the limits as listed in Table 2. The assay value for the marketed formulation was found to be within the limits as listed in Table 2. The low RSD value indicated the suitability of the method for routine analysis of olmesartan medoxomil and hydrochlorothiazide in pharmaceutical dosage forms.

The developed HPTLC technique is simple, precise, specific and accurate and the statistical analysis proved that method is reproducible and selective for the analysis of olmesartan medoxomil and hydrochlorothiazide in bulk drug and tablet formulations.

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